American Journal of Hematology 58:195–199 (1998)

Elevated Plasma Endothelin-1 Levels in Sickle Cell Anemia: Relationships to Oxygen Saturation and Left Ventricular Hypertrophy

Steven G. Werdehoff, R. Blaine Moore, Charles J. Hoff, Elizabeth Fillingim, and Anne M. Hackman*

Department of Pediatrics, University of South Alabama, Mobile, Alabama

Endothelin-1 (Et-1) is a vasoconstrictor produced by endothelial and vascular smooth muscle cells in response to hypoxia, which induces hypertrophy in cultured cardiac myocytes. We measured plasma Et-1 levels and left ventricular dimensions in 13 patients with sickle cell anemia (SCD) and in 12 African-American controls ages 16–29 years. Endothelin-1 concentrations are significantly higher in SCD subjects than controls (10.6 ± 1.9 vs. 3.0 ± 1.3 pmol/L). There was a negative correlation between oxygen saturation and Et-1 levels in SCD patients ($r = -0.71$, $P = 0.01$). SCD subjects have more dilated and hypertrophied hearts corrected for body surface area than controls as evidenced by significant increases in left ventricular end diastolic dimension (31 ± 0.8 vs. 24 ± 0.9 mm/m^2, $P < 0.001$), left ventricular end systolic dimension (20 ± 0.9 vs. 16 ± 0.8 mm/m^2, $P = 0.002$), left ventricular posterior wall thickness (5.0 ± 0.1 vs. 4.0 ± 0.1 mm/m^2, $P < 0.001$), and left ventricular mass (125 ± 7.2 vs. 69 ± 5.1 g/m^2, $P < 0.001$). The index of left ventricular function, the shortening fraction, was not different between groups (34 ± 1.2% in SCD vs. 35 ± 1.5% in controls). The correlation between left ventricular mass and levels of Et-1 in SCD subjects was not significant ($r = 0.47$, $P = 0.121$). Am. J. Hematol. 58:195–199, 1998.

Key words: endothelin-1; sickle cell anemia; cardiac hypertrophy; echocardiography; hypoxia

INTRODUCTION

Sickle cell anemia (SCD) is caused by a point mutation that results in an amino acid substitution (Glu ⇒ Val) in the sixth position of the β chain of hemoglobin. This change in hemoglobin causes red blood cell deformation, resulting in occlusion of small vessels and local tissue hypoxia and damage. The disease is marked by painful vaso-occlusive episodes that cause systemic organ damage, with death usually resulting from sepsis or renal failure. Between 10 and 30% of SCD patients die primarily from congestive heart failure [1]. Left ventricular hypertrophy, one of the precursors of heart failure, has been documented in adults and children with SCD [2,3].

Endothelin-1 (Et-1) is a 21-amino acid peptide that is the most potent endogenous vasoconstrictor discovered to date [4]. It is produced primarily by endothelial cells and binds to specific receptors on vascular smooth muscle cells. Binding of Et-1 to type A endothelin receptors results in vasoconstriction due to an increase in intracellular calcium that is mediated by activation of phospholipase C [4]. Et-1 has also been shown to stimulate mitogenic activity in cardiac myocytes in vitro through activation of protein kinase C [4,5]. Banding the ascending aorta in rats results in a significant increase in serum and left ventricular levels of Et-1 and rapid development of left ventricular hypertrophy [6]. Pretreatment with an antagonist to the type A endothelin receptor prevents the rapid development of hypertrophy. Although the mechanism of Et-1 release in response to pressure overload is unknown, these observations suggest that Et-1 is an important cellular signal in the development of

*Correspondence to: Anne M. Hackman, M.D., Pediatric Cardiology, 3 Mobile Infirmary Circle, Suite 305, Mobile, AL 36607.

Received for publication 4 November 1997; Accepted 18 March 1998

cardiac hypertrophy. Other conditions that cause an increase in Et-1 levels include hypoxemia and shear stress [4,7,8].

SCD patients are subject to chronic hypoxia due to low hemoglobin and sickling of red blood cells [9]. Sickled erythrocytes may also subject endothelial cells to shear stress due to their poor distensability and adhesion to the endothelium. These conditions are likely to result in an elevated plasma concentration of Et-1. An in vitro study of human endothelial cells exposed to sickled erythrocytes showed a 4- to 8-fold increase in transcription of the Et-1 gene [10].

The purpose of this study was to determine whether plasma levels of Et-1 are increased in young adults with sickle cell anemia compared to African Americans of similar age without SCD. Secondly, we wanted to determine if there was a correlation between decreased oxygen saturation and increased Et-1 concentrations. Furthermore, we sought to confirm that the left ventricles of SCD patients are dilated and hypertrophied as compared to healthy controls. Finally, we wanted to assess whether there is a positive correlation between Et-1 concentration and the degree of left ventricular hypertrophy.

MATERIALS AND METHODS

SCD subjects between the ages of 16 and 30 years were recruited from a university sickle cell anemia clinic. Healthy control subjects were recruited from the local African-American population to approximate the age and sex distribution of the SCD subjects. SCD subjects were excluded if they had any signs or symptoms of a pain crisis at enrollment, or if they had been hospitalized or transfused within the past 3 months. After informed consent and ethics committee approval was obtained, venous blood was drawn from each subject in the supine position into tubes containing EDTA. Blood could not be obtained from 1 of the SCD subjects. Blood samples were placed on ice prior to centrifugation for 15 min at 4°C and 2,000*g*. Plasma was removed and stored at −70°C. Hemoglobin and hematocrit were measured from a 1-ml aliquot of blood that was removed prior to centrifugation. Duplicate samples of plasma from each subject were eluted through a solid phase extraction on C2 silica columns. Et-1 concentrations were measured by a commercially available ELISA kit (Amersham Life Sciences, Buckinghamshire, England). The mean of duplicate measurements is reported. Six subjects had more than 2 measurements performed. The Et-1 concentration for these 6 patients represents the mean of all measurements. Sensitivity to Et-1 was reported by the manufacturer to be 0.6 pmol/L. Nonspecific binding in the absence of Et-1 was 5.9%. Cross-reactivity with endothelin-3 was less than 0.001%.

An echocardiogram was performed on all participants except for two control subjects. Standard M-mode echocardiography from the parasternal short axis view was used to measure left ventricular end diastolic dimension (LVED), left ventricular end systolic dimension (LVES), and left ventricular posterior wall dimension (LVPWD) in diastole [11]. All measurements were made at the level of the papillary muscles and indexed to body surface area. Measurements were made by two separate observers who were blinded to the clinical status of the subjects. Two parameters were calculated from the M-mode measurements:

$$\text{Shortening fraction (\%)} = \frac{LVED - LVES}{LVED} \times 100$$

$$\text{Left ventricular mass (g)} = 1.04\,[(LVED + 2LVPWD)^3 - LVED^3] - 14.$$

Shortening fraction (SF) is an estimate of left ventricular systolic function and left ventricular mass (LVM) is an estimate of the weight of the heart [12], which was indexed to body surface area (LVMI in g/m^2).

Statistical analysis of the data was performed using Systat for Windows software [13]. Unpaired student's *t*-tests were used to determine differences between SCD subjects and controls for all parameters. A P value < 0.05 was considered significant. Group values are reported as means ± SEM. Pearson correlation coefficients were used to determine whether there was any correlation between plasma Et-1 concentrations and both LVMI and oxygen saturation in the SCD subjects.

TABLE I. Baseline Characteristics of Subjects*

Parameter	SCD (n = 13)[a]	Control (n = 12)[a]	*P* value
Age (years)	21 ± 1	23 ± 1	0.403
Sex	8 F, 5 M	6 F, 6 M	NS
Height (cm)	171 ± 3	169 ± 2	0.490
Weight (kg)	69 ± 2	87 ± 10	0.100
O_2 saturation (%)	94 ± 1	97 ± 0.2	0.014
Hemoglobin (g/dl)	8.5 ± 0.3	13.2 ± 0.3	<0.001
Hematocrit (%)	27 ± 1	42 ± 1	<0.001

*SCD, sickle cell anemia; F, female; M, male; NS, not significant.
[a]Values represent the mean ± SEM.

RESULTS

Baseline characteristics of the two subject groups are given in Table I. The mean age of the SCD subjects (5 men and 8 women) was 21 ± 1 years with a range of 16–29. Control subjects (6 men and 6 women) had a mean age of 23 ± 1 years with a range of 18–29. There was no significant difference between the two groups with respect to height and weight. Sickle cell subjects

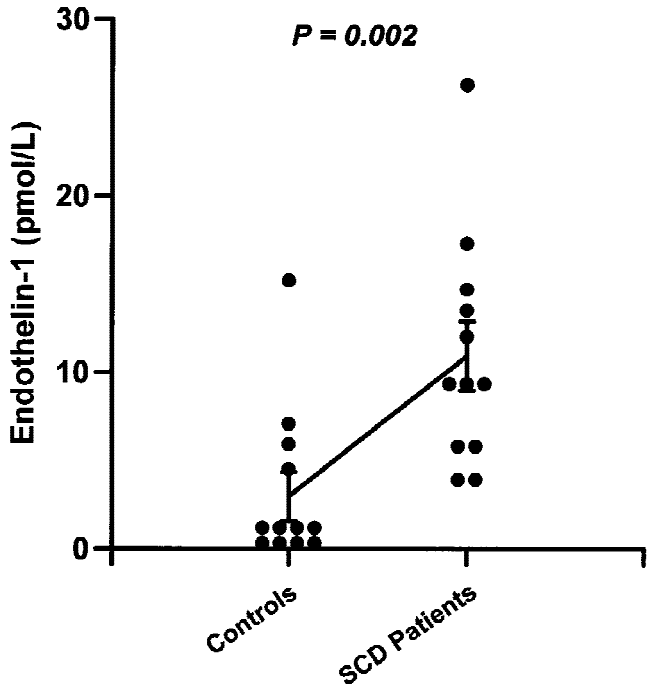

Fig. 1. Plasma endothelin-1 concentration was significantly greater in subjects with sickle cell anemia (SCD patients) compared with control subjects. Values are means ± SEM. Means of the two groups are connected by a line for clarity.

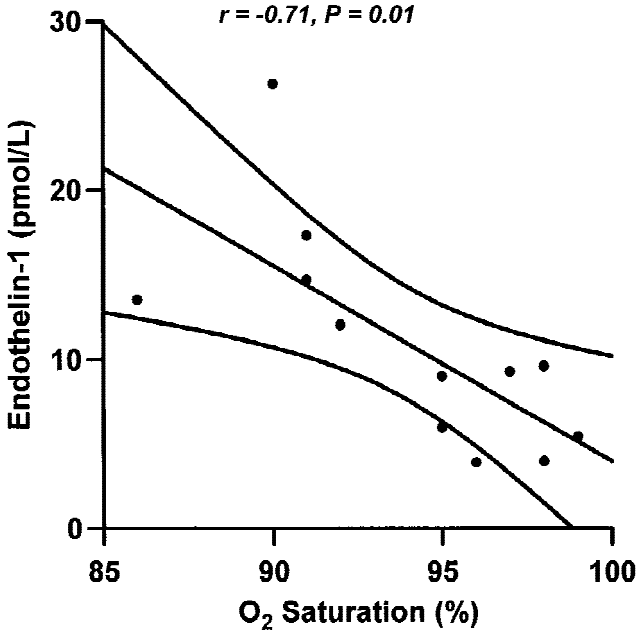

Fig. 2. Pearson correlation with a 95% confidence interval is depicted above between endothelin-1 concentration and oxygen saturation for the subjects with sickle cell disease. There is a significant negative correlation between endothelin-1 concentration and oxygen saturation.

had significantly lower hemoglobin, hematocrit, and oxygen saturation compared with controls.

Plasma Et-1 concentrations were significantly increased in subjects with SCD (Fig. 1). SCD subjects had a mean Et-1 concentration of 10.9 ± 1.9 pmol/L and the control group had a mean concentration of 3.0 ± 1.3 pmol/L ($P = 0.002$). The mean coefficient of variation between duplicate measurements was 0.22 ± 0.04. There was a negative correlation between plasma levels of Et-1 and oxygen saturation in SCD subjects with a correlation coefficient of $r = -0.71$ $P = 0.01$) (Fig. 2).

The left ventricles of the SCD subjects were more dilated and hypertrophied than control subjects (Fig. 3a,b). SCD subjects had significantly greater LVED index (31 ± 0.8 vs. 24 ± 0.9 mm/m^2, $P < 0.001$), LVES index (20 ± 0.9 vs. 16 ± 0.8 mm/m^2, $P = 0.002$), and LVPWD index (5.0 ± 0.1 vs. 4.0 ± 0.1 mm/m^2, $P < 0.001$) compared with control subjects. Calculated left ventricular mass indexed to body surface area (LVMI) was also increased in SCD subjects (125 ± 7.2 vs. 69 ± 5.1 g/m^2, $P < 0.001$) (Fig. 4). The measurement of left ventricular function, the shortening fraction, was not significantly different between the two groups (34 ± 1.2% in SCD vs. 35 ± 1.5% in controls). Interobserver variability between 2 observers blinded to the clinical status of the subjects for all M-mode measurements was less than 10%.

No significant correlation was found between Et-1 levels and LVEDI, LVESI, or LVPWDI in the SCD patients. Although it did not reach statistical significance, there was a weak positive correlation between LVMI and Et-1 levels in the SCD patients ($r = 0.47$, $P = 0.121$) (Fig. 5).

DISCUSSION

Our results are similar to those in previous studies that have demonstrated left ventricular dilation and hypertrophy in SCD patients with no apparent loss of function [2,3]. While no symptoms of cardiac dysfunction have developed in these patients, the subclinical changes in the left ventricle may later play a role in cardiac complications such as congestive heart failure. Et-1 has been shown to be an important growth factor for cardiac myocytes in vitro and to be at increased concentrations in the serum of animals during the development of cardiac hypertrophy in vivo [5,6]. Plasma Et-1 concentrations have also been reported to correlate with the severity of diseases such as congestive heart failure in humans [4]. Although our results did not demonstrate a significant correlation between plasma Et-1 concentration and the degree of left ventricular hypertrophy, such an association still may exist. Because left ventricular hypertrophy develops over a number of years, while the plasma half-life of Et-1 is 4–7 min, serial measurements of plasma

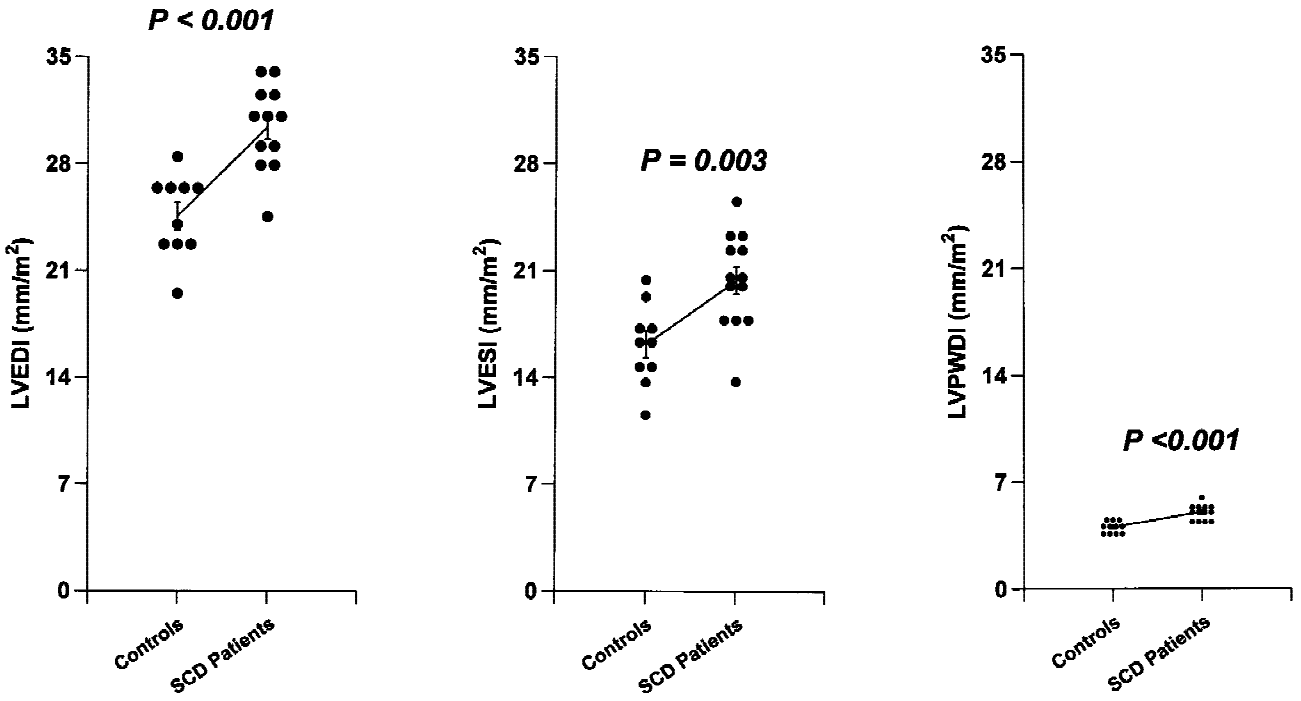

Fig. 3. The results of standard M-mode echocardiography indexed to body surface area for the two study groups are depicted above as group means ± SEM. Means of the two groups are connected by a line for clarity. Patients with sickle cell anemia (SCD patients) had significantly greater left ventricular end diastolic dimension index (LVEDI), left ventricular end systolic dimension index (LVESI), and left ventricular posterior wall dimension index (LVPWDI) compared with controls.

Et-1 over time might be necessary to elucidate a correlation with left ventricular hypertrophy in SCD if it exists.

Our results indicate that plasma Et-1 levels are significantly increased in young adults with SCD. This is presumably due to chronic conditions of hypoxemia and shear stress inherent in SCD. Other studies have shown a strong correlation between hypoxemia and Et-1 levels in vitro and in vivo [7,8]. Our results also demonstrate a correlation between low oxygen saturation and increased Et-1 concentrations in patients with SCD. The chronic effect of endothelial shear stress on plasma Et-1 levels in SCD subjects is difficult to assess in vivo, but it may contribute significantly to their increased baseline levels. Sickled cells applied to endothelial cells in vitro have been shown to cause increased production of Et-1 [10]. If this is true in vivo, then the vasoconstriction produced by increased Et-1 could play a role in promoting vaso-occlusive episodes, which are believed to the underlying cause of the systemic organ damage prevalent in SCD patients. A small study of two patients with sickle cell disease by Hammerman et al. [14] supports this hypothesis. They found Et-1 levels were elevated during acute chest syndrome, less elevated during recovery, and only mildly increased during a subsequent clinic visit in the same two subjects followed longitudinally. Additional studies are necessary to evaluate Et-1 levels in SCD patients at baseline and during crises. A recent study in

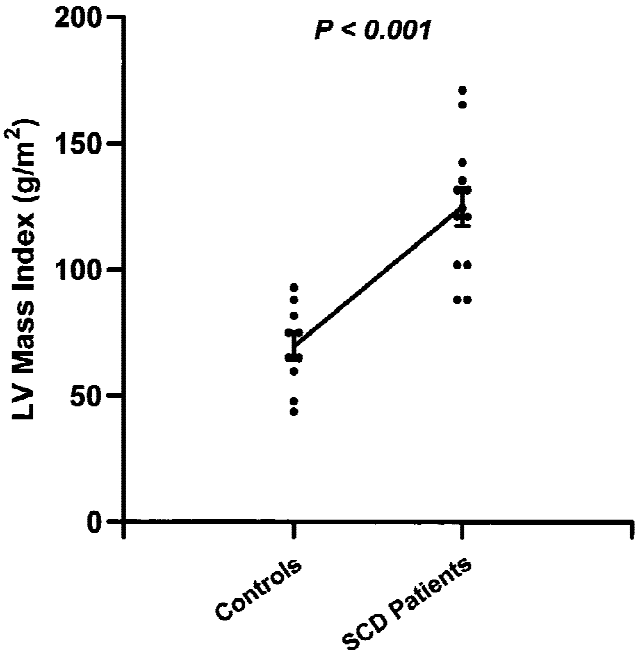

Fig. 4. Calculated left ventricular mass indexed to body surface area (LVMI) was increased significantly in subjects with sickle cell anemia (SCD patients) vs. control subjects. Values are means ± SEM. Means of the two groups are connected by a line for clarity.

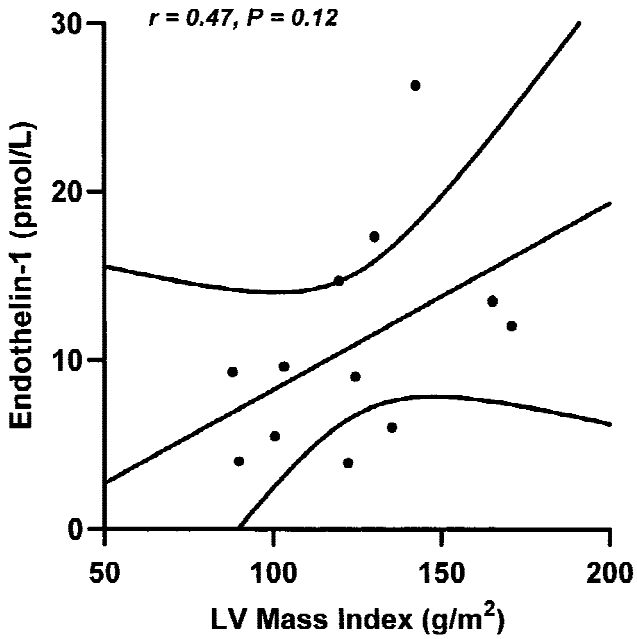

Fig. 5. Pearson correlation with a 95% confidence interval is depicted above between endothelin-1 concentration and left ventricular mass index (LVMI) for the subjects with sickle cell disease. There is a trend towards a positive association between endothelin-1 concentration and LVMI.

healthy men demonstrated vasodilation and decreased peripheral vascular resistance following intravenous administration of an Et-1 receptor antagonist [15]. If levels of Et-1 are consistently elevated in SCD patients during crises, an Et-1 receptor antagonist might be beneficial in the treatment of these episodes since it would be expected to prevent vasoconstriction.

CONCLUSIONS

Plasma endothelin-1 levels are elevated in young adults with sickle cell anemia. There is a negative correlation between oxygen saturation and Et-1 concentrations in sickle cell subjects. We did not find a correlation between Et-1 levels and left ventricular chamber size, wall thickness, or calculated left ventricular mass in subjects with sickle cell anemia.

REFERENCES

1. Falk RH, Hood WB: The heart in sickle cell anemia. Arch Int Med 142:1680, 1982.
2. Gerry JL, Baird MG, Fortuin NJ: Evaluation of left ventricular function in patients with sicke cell anemia. Am J Med 60:968, 1976.
3. Rees AH, Stefadouros MA, Strong WB, Miller MD, Gilman P, Rigby JA, McFarlane J: Left ventricular performance in children with homozygous sickle cell anemia. Br Heart J 40:690, 1978.
4. Levin ER: Mechanisms of disease: Endothelins. N Engl J Med 333:356, 1995.
5. Shubeita HE, McDonough PM, Harris AN, Knowlton KU, Glembotski CC, Brown JH, Chien KR: Endothelin induction of inositol phospholipid hydrolysis, sarcomere assembly, and cardiac gene expression in ventricular myocytes. A paracrine mechanism for myocardial cell hypertrophy. J Biol Chem 33:20555, 1990.
6. Ito H, Hiroe M, Hirata Y, Fujisaki H, Adachi S, Akimoto H, Ohta Y, Marumo F: Endothelin ETA receptor antagonist blocks cardiac hypertrophy provoked by hemodynamic overload. Circulation 89:2198, 1994.
7. Kagumu H, Suzuki T, Arakawa M, Mitsui Y: Low oxygen enhances endothelin-1 (Et-1) production and responsiveness to Et-1 in cultured cardiac myocytes. Biochem Biophys Res Commun 202:1612, 1994.
8. Ferri C, Bellini C, De Angelis C, De Siati L, Perrone A, Properzil G, Santucci A: Circulating endothelin-1 concentrations in patients with chronic hypoxia. J Clin Pathol 48:519, 1995.
9. Noguchi CT, Schechter AN, Rodgers GP: Sickle cell disease pathophysiology. Bailliere's Clin Haematol 6:57, 1993.
10. Phelan M, Perrine SP, Brauer M, Faller DV: Sickle erythrocytes, after sickling, regulate the expression of the endothelin-1 gene and protein in human endothelial cells in culture. J Clin Invest 96:1145, 1995.
11. Snider AR, Serwer GA, Ritter SB: Echocardiography in Pediatric Heart Disease, 2nd ed. St. Louis: Mosby-Year Book, Inc., 1997.
12. Devereux RB, Reichek N: Echocardiographic determination of left ventricular mass in man. Anatomic validation of the method. Circulation 55:613, 1977.
13. Wilkinson L: SYSTAT 6.0 for Windows. Chicago, IL: Statistics SSPS, Inc., 1996.
14. Hammerman SI, Kourembanas S, Conca TJ, Tucci M, Brauer M, Farber HW: Endothelin-1 production during the acute chest syndrome in sickle cell disease. Am J Respir Crit Care Med 156:280, 1997.
15. Haynes WG, Ferro CJ, O'Kane KP, Somerville D, Lomax CC, Webb DJ: Systemic endothelin receptor blockade decreases peripheral vascular resistance and blood pressure in humans. Circulation 93:1860, 1996.